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Parasites and Wildlifejournal homepage: [www.elsevier.com/locate/ijppaw](http://www.elsevier.com/locate/ijppaw)Sentinels in a climatic outpost: Endoparasites in the introduced muskox (*Ovibos moschatus wardi*) population of Dovrefjell, NorwayRebecca K. Davidson<sup>a,\*</sup>, Hanne Amundsen<sup>b</sup>, Nora Oftenes Lie<sup>b</sup>, Katrien Luyckx<sup>b,1</sup>, Lucy J. Robertson<sup>b</sup>, Guilherme G. Verocai<sup>c</sup>, Susan J. Kutz<sup>c,d</sup>, Bjørnar Ytrehus<sup>a,2</sup><sup>a</sup> Norwegian Veterinary Institute, Postboks 750 Sentrum, 0106 Oslo, Norway<sup>b</sup> Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Postboks 8146 Dep, 0033 Oslo, Norway<sup>c</sup> Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada<sup>d</sup> Canadian Cooperative Wildlife Health Centre – Alberta Node, Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada

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## ABSTRACT

We assessed the occurrence of endoparasite eggs, cysts, oocysts and larvae in the muskox population of Dovrefjell, Norway, during June and August 2012. This population originates from 13 calves translocated from Eastern Greenland during the 1950s. A total of 167 faecal samples were collected, of which 49% came from identified individuals: 165 were examined by the Baermann and 95 by McMaster techniques and 167 by immunofluorescence antibody test (IFAT). Lungworm larvae recovered in the Baermanns were identified as Protostrongylidae (82%) and *Dictyocaulus* sp. (76%) based on morphology. Further molecular analyses of the ITS-2 region of two protostrongylid larvae from two muskoxen as *Muellerius capillaris*. Larval prevalence and intensity differed significantly between samples collected from the different age groups in June and August, with increasing prevalence and intensity in calves during the course of their first summer, whereas intensity decreased in adults from June to August. McMaster test and IFAT were used to determine the occurrence of infections with intestinal strongyles (84%), *Moniezia* spp. (24%), *Nematodirus* sp. (2%), *Eimeria* spp. (98%), *Cryptosporidium* sp. (14%) and *Giardia duodenalis* (7%). Molecular analyses of three isolates of *Cryptosporidium* and *Giardia* were identified as *Cryptosporidium xiaoi* and *G. duodenalis* assemblage A. Although infection intensity of all these intestinal parasites tended to be low, the high level of polyparasitism, together with the other challenges faced by this population living at the edge of their climatic range, means that these infections should not be ignored. The potential that *M. capillaris*, *Cryptosporidium* and *Giardia* infections derive from other sympatric host species (sheep and reindeer) is discussed.

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## 1. Introduction

In the early and mid-20th century, muskoxen (*Ovibos moschatus wardi*) were repeatedly introduced to Norway from east Greenland (Alendal and Helle, 1983). The current population in Dovrefjell national park, Norway, originated from 13 calves that survived these translocations from Eastern Greenland in the 1950's (Ytrehus et al., 2008). The current muskox population ranges across approximately 340 km<sup>2</sup>, but the animals use different parts of this area depending on season, causing local, temporal high densities. The summer population during 2012 was estimated to be

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*Eimeria* of at least 6 different species), have been reported from wild populations of this host in Arctic North America and Greenland (Kutz et al., 2012). Gastrointestinal helminths of 17 muskoxen from the Dovrefjell region that had been killed by lightning (12 animals), shot (4) or perished due to other misadventure (2) were identified during post mortem examinations in 1970's (Alendal and Helle, 1983). Various nematodes including *Ostertagia* sp., *Marshallagia* sp., *Teladorsagia* sp., *Trichostrongylus* sp., *Cooperia oncophora*, *Nematodirella* sp., *Nematodirus* spp., *Chabertia ovina*, *Trichuris* sp. and the cestode *Moniezia* sp. were reported. *C. ovina* and *Teladorsagia* (reported as *Ostertagia*) *circumcincta* were reported from most animals and both are common parasites of domestic sheep. However, the report of *T. circumcincta* should be revisited as, *Teladorsagia boreoarcticus*, is morphologically very similar to *T. circumcincta* and has since been described as a common parasite of muskoxen across its natural range (Hoberg et al., 1999; Kutz et al., 2012). Alendal and Helle (1983) also reported a high abundance of *Marshallagia marshalli* and *Nematodirella longisimespiculata*, common parasites of North American muskoxen (Kutz et al., 2012), in some animals. Their material was not examined for intestinal protozoan parasites.

Dovrefjell has a warmer summers and winters than Arctic regions with endemic muskox populations. This mountain region is at the border between Arctic and cold temperate climates, the landscape is classified as low to high alpine zones depending on altitude (Michelsen et al., 2011), and is thus a climatic outpost for the Arctic-adapted muskox. These muskoxen may therefore serve as a sentinel population to detect and predict the responses of this Arctic species and its parasites in a changing global climate. The goal of the current study was to provide baseline information on the fauna, prevalence, and intensity of endoparasites among different age classes of the Dovrefjell muskox population during the summer of 2012. Two periods of sampling were carried out, the first in early summer (June) prior to the muskoxen moving to cooler altitudes in the high mountains and the second in late summer whilst muskoxen were still in the higher mountain regions (August). The aim was to update baseline knowledge, as well as investigate lungworm transmission dynamics during the short summer season, to facilitate future research.

## 2. Materials and methods

### 2.1. Sample collection

Muskoxen were located within their grazing area based on the national park ranger knowledge. Animals were observed from a distance of 50–150 m using binoculars, and gender and age

collected during two periods in summer 2012: 96 faecal samples in June (16th–20th June) and 71 in August (13th–16th August).

### 2.2. Analysis of samples for detection and enumeration of endoparasites

A modified McMaster technique, using 3 g of faeces that were homogenised and subsequently suspended in zinc chloride-saline flotation fluid (specific gravity of 1.3), was used for detection and quantification of helminth eggs and *Eimeria* oocysts (Taylor et al., 2007). Egg morphology was used to distinguish between *Moniezia benedeni* and *Moniezia expansa* (Gibbons et al., 2014). The number of eggs counted in two Whitlock Universal chambers (total volume examined 1 mL) was multiplied by 20 to give the overall oocyst per gram (OPG) and egg per gram (EPG) results.

Detection and quantification of *Cryptosporidium* and *Giardia* was done by standard immunofluorescent antibody test (IFAT) on 15 µL sub-samples that had been prepared by re-suspending 3 g of faeces in water followed by sieving and centrifugation. These sub-samples were air-dried, methanol-fixed, stained with FITC-labelled monoclonal antibody cocktail against *Cryptosporidium* oocysts and *Giardia* cysts (Mab: Aqua-Glo, Waterborne Inc., New Orleans, USA) and 4',6-diamidino-2-phenyl indole (DAPI), and examined by fluorescence microscopy. Each sample was scored for *Cryptosporidium* and *Giardia* as either negative (no parasites detected in the sample), +, ++, or +++ as described in a previous publication (Robertson et al., 2010).

An adapted Baermann technique, using 10 g faecal samples wrapped in a double gauze layer and suspended in a water-filled clear plastic bag that was hung diagonally so that larvae sank to one corner of the bag, was used to isolate larvae under field conditions. After 16–24 h the bag was clamped off above the sediment that had collected in the bottom corner and this sediment was collected into a 15 mL tube. The supernatant was aspirated to the 1 mL mark, after the tube had been centrifuged at 1600G for 3 min. A 100 µL homogenised subsample of the sediment was examined for larvae, a further 100 µL taken from the base of the tube was examined if the first subsample did not contain larvae. Ethanol (75%) was added to the remaining sediment, to the 15 mL mark, and stored refrigerated (4 ± 2 °C) until molecular analysis could be carried out. Larvae were examined microscopically at 100× and morphology identified as: protostrongylid larvae with dorsal spine (DSL), *Dictyoacaulus* sp. larvae and others (larvae from hatched strongyle-type eggs or free-living larvae from the environment). The number of each type of larva was recorded for individual samples and the number of larvae per gram (LPG) was calculated by:

$$\frac{\text{Number of larvae detected} \times (\text{Total volume of fluid } (\mu\text{L})) / \text{Volume of subsample examined } (\mu\text{L})}{\text{Weight of original faecal sample (grams)}}$$

determined of individual animals based on the development of the horns and bos (Bretten, 1990; Smith et al., 2008). Observation of defecation was recorded such that faecal samples could be linked to animals of known age class (calf, yearling, young [2–3 years old], adult [4 years old and above]), and sex, with one spotter remaining in position and guiding the sampler, via walkie-talkie, to the “identified” faeces. The faeces was subsequently collected and placed in appropriately labelled zip-lock bags. Faecal samples for which information on the animal could not be determined were marked as unknown age and sex. Faecal samples were

### 2.3. Molecular identification of DSL

Selected DSL had their DNA (gDNA) extracted according to Verocai et al. (2013). PCR was performed using primers NC1 (5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (5'-TTAGTTTCTTTTCTCCGCT-3') targeting the ITS-2 region of rRNA gene as per Verocai et al. (2013). Successfully amplified DSL were column purified using e.Z.N.A MicroElute® Cycle Pure Kit (Omega Bio-Tek) according to the producer's recommendations. Purified PCR products were then sequenced from both ends using the above

mentioned primers with BigDye Terminator Cycle Sequencing (Applied Biosystems). Sequences were edited using MEGA 6 (Tamura et al., 2013). BLAST searches were used to compare the resulting sequences to ITS-2 rRNA sequences available in GenBank, and aligned using Geneious (Drummond et al., 2011). Sequences were deposited in GenBank.

#### 2.4. Analysis of species/genotype for *Cryptosporidium* and *Giardia*

Samples were selected for genotyping on the basis of microscopy results; one of the *Giardia* samples scored ++, and for all samples selected some parasites were nucleated (included DAPI in the nuclei).

The centrifuge pellets of these samples (retained refrigerated) were re-suspended in 1.5 mL water, and transferred to a microcentrifuge tube. The *Cryptosporidium* oocysts and/or *Giardia* cysts were isolated by a modified immunomagnetic separation procedure, as previously published (Robertson et al., 2006), using 15 µL of beads coated with the relevant monoclonal antibody (GC-Combo, Invitrogen). The isolated parasites were then re-suspended in 100 µL Tris–EDTA buffer and placed for one hour in a heat block set at 100 °C for *Cryptosporidium* oocysts and 90 °C for *Giardia*. DNA was then isolated using a QIAmp DNA mini-kit (QIAGEN GmbH, Germany) following the manufacturer's protocol.

For *Giardia* samples, PCR was run at both the glutamate dehydrogenase (gdh) gene (approximately 460 bp) and the β-giardin gene (approximately 515 bp). For *Cryptosporidium* isolates, PCR was targeted at the SSU rRNA gene (approximately 800 bp). Published primers and protocols were used with slight modifications (Xiao et al., 1999, 2nd PCR only of nested protocol; Read et al., 2004; Lalle et al., 2004). PCR amplification products from positive samples were purified (High Pure PCR product purification kit, Roche Applied Science) according to the manufacturer's protocol, and sequenced on both strands at a commercial facility (Eurofins MWG Operon, Germany). Chromatograms and sequences were examined using Chromas and DNA Baser. Consensus sequences were constructed and compared using BioEdit (<http://www.mbio.ncsu.edu/BioEdit/page2.html>).

Sequence searches were conducted using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the sequences obtained in this study compared with sequences published in GenBank.

#### 2.5. Statistical analyses

Statistical analyses were conducted using JMP statistical software package (V11.0.0 SAS Institute Inc.). These included summary statistics and comparisons of prevalence and parasite abundance by age class and sampling month. Contingency analysis was used to look at endoparasite prevalence by age class and the likelihood ratio used to assess significance. Fisher's exact tests (both one tailed and two tailed when relevant) were used to compare prevalence within each age category between the two sampling months. Non-parametric tests were used to analyse the egg, oocyst and larval counts compared to age class, and unless stated otherwise Wilcoxon/Kruskal–Wallis tests (rank sums) were used to compare faecal egg/oocyst intensity between the age classes and pairwise non-parametric comparisons for age classes were made using the Wilcoxon method. A significance level of 5% was selected for analysis purposes.

### 3. Results

#### 3.1. Sample material

A total of 96 faecal samples were collected in June and 71 in August. The distribution of these samples by animal age, sex, and

**Table 1**

The age and sex class of the muskoxen sampled in summer 2012, in Dovrefjell national park, as well as the number of samples analysed using the different methods: Baermann, McMaster and IFAT.

Analysis method	Baermann and IFAT	McMaster
Sex		
Male	16	11
Female	31	19
Unclassified	120	65
Age class		
Calf	19	7
Yearlings	13	7
Young	19	19
Adult	31	14
Unclassified	85	48
Total	167	95

the laboratory analyses run are summarized in Table 1. Investigation of intestinal helminth eggs and coccidia was only carried out on the faecal samples collected in June.

#### 3.2. Intestinal helminths

Strongyle-type eggs; encompassing eggs from the superfamilies Trichostrongyloidea (with the exception of eggs from genus *Nematodirus* and *Marshallagia* and *Nematodirella* which can be morphologically differentiated from the other strongyle-type eggs) and Strongyloidea (Genus Chabertiidae) were detected in all age groups, with an overall prevalence of 84%, but with increasing prevalence with increasing age ( $p < 0.001$ ) (Table 2). Egg intensity (EPG) was significantly affected by age class ( $p < 0.001$ ) with egg counts significantly lower in calves compared with all other age groups ( $p < 0.001$ ). Yearlings and young (2–3 year old) animals had the highest infection intensities (as demonstrated by egg counts), with egg counts significantly higher in young animals than in adults ( $p = 0.001$ ). The maximum infection intensity was 280 eggs per gram (EPG) in a faecal sample from the unknown age group.

*Nematodirus* sp. (but not *Nematodirus battus*) was detected in two faecal samples (2% prevalence) at very low infection levels (both at 20 EPG). In both cases the samples came from animals in the young age class. Eggs of *Nematodirella* sp., *Marshallagia* sp. and *Trichuris* sp. were not detected.

Anoplocephalid cestodes were detected in 23 animals (prevalence of 26%), with *M. expansa* detected more frequently than *M. benedeni*. *M. expansa* was detected in 11 samples, *M. benedeni* in six, whilst five samples had both species and in one sample it was not possible to differentiate which species was present. *Moniezia* was detected more frequently in young animals than the other age classes ( $p = 0.034$ ).

#### 3.3. Intestinal protozoa

The prevalence of *Eimeria* spp. infection was high, with all samples analysed being positive, apart from samples from two calves (prevalence of 98%). No significant differences in oocyst intensity were seen between the age classes however the two highest outliers both came from samples from calves and had more than 30,000 OPG. No attempt was made to identify *Eimeria* infections to species level although morphological appearance suggested the presence of more than one species.

*Cryptosporidium* oocysts were detected in 17 samples (10%) and *Giardia* cysts were detected in 7 samples (4%). All *Cryptosporidium* positive samples were scored as + (on a scale of 0 to +++), with most having no more than a few parasites per sub-sample. At least one sample from each age group was positive for *Cryptosporidium*

**Table 2**

Prevalence and mean shedding intensity of endoparasite eggs (EPG) and oocysts (OPG) in muskoxen in Dovrefjell national park, Norway in June 2012. The prevalence (with 95% confidence interval (CI)), and where relevant mean EPG/OPG count (with 95% CI) and median EPG/OPG is shown for each endoparasite identified using McMaster's egg flotation method or IFAT (for *Giardia* sp. and *Cryptosporidium* sp.). In addition to the endoparasites listed in the table *Nematodirus* sp. eggs were detected in two faecal samples from the young age class (2–3 year olds) at low levels (20 EPG). *Marshallagia* sp., *Trichuris* sp. and *Nematodirella* sp. eggs were not detected in any of the samples.

Age	N (IFAT)	P% [95% CI] Mean EPG/OPG (Median)	Strongyle type eggs	<i>Moniezia</i> spp.	<i>Eimeria</i> sp.	<i>Giardia</i> sp.	<i>Cryptosporidium</i> sp.
Calf	7 (19)	P EPG/OPG [95%CI] (M)	14.3% [2.6–51.3] 1.4 [–2.1–4.9] <sup>a</sup> (0)	0.0% [0–32.4]NA	71.4% [35.9–91.8] 10,238 [–4768–6133] (1350)	0.0% [0–16.8] NA <sup>1</sup>	15.8% [5.5–37.6] NA
Yearling	7 (13)	P EPG/OPG [95%CI] (M)	85.7% [48.7–97.4] 78.6 [–0.2–157.3] (40)	28.6% [8.2–64.1] NA	100% [64.6–100] 2343 [260–4426] (1860)	15.4% [4.3–42.2] NA	30.7% [12.7–57.6] NA
2–3 year old (young)	19 (19)	P EPG/OPG [95%CI] (M)	94.7% [75.4–99.1] 83.2 [55.1–111.2] <sup>b</sup> (70)	47.4% [27.3–68.3] <sup>2</sup> NA	100% [83.2–100] 1087 [740–1434] (1020)	0.0% [0–16.8] NA	15.8% [5.5–37.6] NA
Adult	14 (31)	P EPG/OPG [95%CI] (M)	92.9% [68.5–98.7] 34.3 [20.4–48.2] (25)	14.3% [4.0–39.9] NA	100% [78.5–100] 2432 [48–4817] (620)	3.2% [0.1–16.2] NA	9.7% [3.3–24.9] NA
Unknown	48 (85)	P EPG/OPG [95%CI] (M)	87.5% [75.3–94.1] 59.6 [40.9–78.2] (40)	20.8% [11.7–34.3] NA	100% [92.6–100] 1972 [1224–2720] (1260)	4.7% [1.8–11.5] NA	4.7% [1.8–11.5] NA
Total	95 (167)	P EPG/OPG [95%CI] (M)	84.2% [75.6–90.2] 57.7 [45.2–70.1] (40)	24.0% [16.5–33.4] <i>M. expansa</i> and <i>M. benedeni</i> detected in single and mixed infections	97.9% [92.6–99.4] 2499 [1423–3576] (1070)	4.2% [2.0–8.4] <i>Giardia duodenalis</i> Assemblage A detected	10.2% [6.5–15.7] <i>C. xiaoi</i> detected

<sup>a</sup> mean EPG significantly lower than in other age classes.

<sup>b</sup> mean EPG significantly higher than in adults.

<sup>1</sup> NA (not applicable).

<sup>2</sup> *Moniezia* had a significantly higher prevalence in young animals than other age classes.

oocysts, but too few samples were found positive to determine any age-distribution pattern. For *Giardia*, positive samples were identified in adults and yearlings.

*Cryptosporidium* oocysts from two samples were successfully amplified at the SSU-rRNA gene, and had 100% sequence identity to *Cryptosporidium xiaoi* sequences in GenBank, including GQ337963 from a Norwegian sheep isolate. Genotyping was successfully performed at both the  $\beta$ -giardin gene and the *gdh* gene for one *Giardia* sample; this was identified as assemblage AI, and had 100% identity to analogous sequences in GenBank, including GQ329671 at the  $\beta$ -giardin gene from a human isolate in Sweden and a portion of the *gdh* gene from the Portland (human) isolate, GenBank Accession number EF685701.

### 3.4. Protostrongylid and *Dictyocaulus* sp. larvae

Protostrongylid DSL and *Dictyocaulus* sp. were detected in 82% and 78% of the faecal samples, respectively (Table 3). Larval intensity in the positive animals ranged from 1 to 265 LPG for DSL (mean 34.4 and median 17.5) and from 0.4 to 760 LPG for *Dictyocaulus* sp. (mean 72.2 and median 24.5). Analysis of the results showed mean *Dictyocaulus* LPG in the dataset as a whole was significantly lower in August than in June ( $p = 0.021$ ) whilst the opposite trend was seen for DSL, although the significance level was not reached ( $p = 0.097$ ). The highest mean *Dictyocaulus* sp. LPG was recorded in yearlings. No significant differences were seen in the dataset as a whole for *Dictyocaulus* ( $p = 0.215$ , 2-tailed test) or DSL ( $p = 0.456$ , 2-tailed test) larval prevalence between the two sampling months but significant age related differences were seen.

Yearlings had significantly higher *Dictyocaulus* larval intensities than the other classes in June ( $p = 0.001$ ). Calves and yearlings had significantly lower DSL larval intensities in June ( $p < 0.001$ ) and August ( $p = 0.003$ ) than the other age classes tested in those periods.

*Dictyocaulus* larval prevalence in calves was higher in August than June although the significance level was not reached

( $p = 0.008$ , right tailed test; greater for August than June) however larval intensity was significantly higher in August than June ( $p = 0.040$ ). Similarly DSL larval prevalence ( $p = 0.007$ , right tailed test) and intensity ( $p = 0.008$ ) were significantly higher in August than June in the calf age group. No significant differences in *Dictyocaulus* or DSL larval prevalence and intensity were seen in yearlings. The age class representing 2–3 year old (young) animals were only sampled in June so further comparison was not possible and no significant differences in larval prevalence and intensity were seen in samples from the unknown age class group. Adults had significantly higher *Dictyocaulus* larval intensity in June compared to August ( $p < 0.001$ ), and larval prevalence was significantly higher in June than August ( $p = 0.017$ , left tailed test; greater for June than August). Adults also had higher DSL intensity in June compared to August ( $p = 0.052$ ) although the prevalence level remained equally high in both periods as all adult samples tested positive for DSL larvae.

We obtained ITS-2 sequences (405 bp) of 2 DSL larvae from 2 different samples from unknown individuals from different locations. Both were identified as *Muellerius capillaris* (GenBank accession number: KJ534589–90), and shared 99–100% identity with *M. capillaris* ITS-2 sequences available in GenBank (AY679327,28,30).

## 4. Discussion

This study provides an overview of endoparasites in the muskox population in Norway and demonstrates that polyparasitism is common. *Muellerius capillaris*, *Cryptosporidium*, *Giardia*, and *Eimeria* spp. are reported for the first time from this population. Previous reports of possible *M. capillaris* infections in Scandinavia were based only on larval morphology (Alendal and Helle, 1983), whereas our data provides unequivocal identification using molecular methods.

*Cryptosporidium* has previously been reported from muskoxen in North America, but not identified to species level (Kutz et al., 2012). *C. xiaoi* was identified in our study; this species is commonly



**Table 3**

Results of Baermann analysis of faecal samples collected from muskoxen in Dovrefjell national park, Norway, in 2012, by age class and month.

Age Group	Number (N) Prevalence (P) Mean LPG (A)	<i>Dictyocaulus</i> sp.		<i>Protostrongylid</i> larvae	
		June	August	June	August
Calves	N	8	11	8	11
	P [95% CI]	13% [2–47]	55% [28–79]	0% [0–32]	64% [35–85] <sup>c</sup>
	A [95% CI]	0.1 [0–0.2]	22 [0–56]	0 [0–0] <sup>b</sup>	10 [2–18] <sup>b,c</sup>
Yearlings	N	7	6	7	6
	P [95% CI]	57% [25–84]	50% [19–81]	27% [8–64]	67% [30–90]
	A [95% CI]	134 [0–336] <sup>a</sup>	3 [0–7]	5 [0–14] <sup>b</sup>	4 [0–10] <sup>b</sup>
2 & 3 year olds	N	17	0	17	0
	P [95% CI]	71% [47–87]		100% [82–100]	
	A [95% CI]	45 [7–84]		24 [15–34]	
Adults	N	14	17	14	17
	P [95% CI]	100% [79–100] <sup>d</sup>	65% [41–83]	100% [79–100]	100% [82–100]
	A [95% CI]	55 [29–82] <sup>d</sup>	6 [2–11]	80 [37–123]	34 [21–47]
Unknown	N	48	37	48	37
	P [95% CI]	92% [80–97]	89% [75–96]	85% [73–93]	92% [79–97]
	A [95% CI]	87 [42–131]	67 [33–101]	18 [9–26]	44 [26–63]
Total	N	94	71	94	71
	P [95% CI]	79% [71–87]	75% [63–83]	79% [69–86]	87% [78–93]
	A [95% CI]	71 [48–93]	38 [12–64]	26 [17–34]	32 [22–42]

<sup>a</sup> Significantly higher mean LPG than other age classes in that month.<sup>b</sup> Significantly lower mean LPG than other age classes in that month.<sup>c</sup> Significantly higher prevalence or mean LPG in August than June.<sup>d</sup> Significantly higher prevalence or mean LPG in June than August.

associated with infection in sheep and goats (Robertson et al., 2014), and has previously been reported from sheep flocks in Norway (Robertson et al., 2010) but does not infect bovine calves (Fayer and Santín, 2009). Thus, these infections may represent spillover from sheep sharing grazing area with the muskoxen. As *Cryptosporidium* species identification was limited to two isolates, other species might also be present. We saw no evidence of clinical disease from *Cryptosporidium* infection and *C. xiaoi* infections in sheep and goats are often asymptomatic, however severe gastrointestinal cryptosporidiosis has been reported from captive muskox calves, although this could be another species (Kutz et al., 2012).

*Giardia duodenalis* (Assemblage A) infection (21% prevalence) has previously been reported from muskoxen on Banks Island, Northwest Territories, Canada (Kutz et al., 2008). Although prevalence in Norwegian muskoxen was lower, it was also Assemblage A, perhaps indicating spillover from humans as was suggested for the Canadian infections. The  $\beta$ -giardin sequence from the muskox isolate in our study had a homology with a human isolate from Sweden, further supporting the possibility of a human source of infection. Previous studies on *Giardia* from sheep in Norway have reported Assemblage E infection (Robertson et al., 2010), but a study on wild reindeer reported Assemblage A (Robertson et al., 2007), suggesting that cross-transmission with sympatric reindeer could also be relevant.

Clinical effects of *Giardia* infection in muskoxen are unknown and bovid infections are often asymptomatic, although decreased weight gain and reduced performance has been reported in cattle (Geurden et al., 2010). It would be interesting to determine whether or not muskoxen are susceptible to infection with *Giardia* of Assemblage E, and whether sheep in this area are infected with *Giardia* and with which genotype.

Lungworms were prevalent in the Dovrefjell muskox population, but interpretation of larval counts is challenging. Levels between 2 and 100 LPG are considered moderate in small ruminants (DTU Veterinærinstituttet, 2009), but whether the same holds true for muskoxen is unresolved. These criteria would suggest moderate infection of the Norwegian muskox population with both *Dictyocaulus* sp. and *M. capillaris*, the exceptions being yearlings in June, which had high levels of infection with *Dictyocaulus*

sp., and calves in June, for which larval levels were very low. Widespread infection with lungworm could render muskoxen more susceptible to other respiratory pathogens; as this population has recently experienced two major respiratory bacterial disease outbreaks (Ytrehus et al., 2008), this could be important. The latest outbreak, with *M. ovipneumoniae* identified, occurred one week after the field sampling for this study (Norwegian Veterinary Institute, 2013).

Lowest *Dictyocaulus* prevalence and intensity were found in calves in June, presumably due to limited exposure prior to June sampling (calving occurs in early May) and the pre-patent period prior to larval excretion, but levels approached those of yearlings by August. Highest *Dictyocaulus* infection intensity was seen in yearlings. Lungworm prevalence increased with age, but infection intensity (LPG) tended to decrease with age. Adults had significantly lower *Dictyocaulus* larval burdens in August than June, suggesting either immunological development, as reported with *Dictyocaulus viviparus* (Hagberg, 2008), or seasonal variation. Arrested larval development of *D. viviparus* has been described in cattle (Gupta and Gibbs, 1970), but whether this occurs in muskoxen is unknown. *Dictyocaulus eckerti* adults have previously been identified at postmortem in muskoxen from this population (unpublished data), North American muskoxen, and also in reindeer (Divina et al., 2002).

Protostrongylid parasites have not been found in the limited surveys of Greenland muskoxen, the original source of the Dovrefjell population (Kutz et al., 2012; Steele et al., 2013), nor, until recently (Kutz et al., 2013), in any high arctic muskox populations (presumably due to absence of gastropod intermediate hosts and low temperatures). Therefore it seems highly improbable that the translocated calves were infected with protostrongylids. *M. capillaris* is a parasite of sheep and goats and has never been reported in cervids; thus, *M. capillaris* in these muskoxen probably demonstrates spillover from domestic sheep. Spillover of other protostrongylids from wild ungulates to translocated muskoxen is reported in North America (e.g., *Protostrongylus stilesi* from Dall's sheep [*Ovis dalli dalli*]; *Varestrongylus* sp. from caribou [*Rangifer tarandus granti* and *R. t. caribou*]) (Hoberg et al., 2002; Kutz et al., 2007) suggesting that muskoxen are vulnerable hosts to this family

of parasites. Sympatric reindeer in Dovrefjell are host to *Elaphostrongylus rangiferi*, which can cause fatal neurological disease in sheep and goats (Handeland, 2002) and probably muskoxen (Holt et al., 1990). However, it is unlikely that *E. rangiferi* would reach patency in muskoxen, and more likely would cause clinical symptoms as seen in other caprines. Other protostrongylids that may occur in Dovrefjell and could infect muskoxen include lungworms belonging to the genus *Varestrongylus* from red deer and moose, or *Elaphostrongylus cervi* or *Elaphostrongylus alces* also from red deer and moose, respectively.

Interpretation of the gastrointestinal parasite egg data from our study is hampered by lack of species identification of the strongyle-type eggs. Unravelling which parasite species native to muskoxen survived the original translocation event and which result from subsequent host-switching is beyond the scope of this study. The absence of *M. marshalli*, which is present in muskoxen in Greenland (Steele, 2013) and previously reported in Norway (Alendal and Helle, 1983), is of note. Given the sample size used in our study and the previous high prevalence reported by Alendal and Helle (1983), sampling bias in the current study is an unlikely explanation for this trend.

*Moniezia* sp. and *M. expansa* infections have been previously reported from muskoxen from North America (Kutz et al., 2012), and also from Dovrefjell (Alendal and Helle, 1983). In this study we identified both *M. expansa* and *M. benedeni*. It is not possible to determine the extent to which these *Moniezia* represent introduction with muskoxen, spillover from other ruminants, or an admixture of these.

*Eimeria* are likely candidates for translocation with muskox calves from Greenland, given the host specificity of this genus and presence in young animals (Samuel and Gray, 1974). The lack of species data in both the Norwegian and Greenlandic muskox populations means that this hypothesis needs further testing.

Age-related differences in intestinal nematode egg prevalence and shedding intensity (egg counts) were seen in this study. Calves had the lowest prevalence and intensity of strongyle-type eggs, presumably due to low exposure and the pre-patent period prior to egg excretion. Furthermore, some strongyle larvae may have entered arrested development as demonstrated for *Ostertagia gruehneri* in caribou at high latitudes (Hoar et al., 2012) and thus not matured until the following year. Overall prevalence increased with age, but as highest infection intensity was in yearlings and young animals, partial development of immunity in older age groups is indicated. Although gastrointestinal nematodes reduce productivity in domestic livestock, our understanding of impacts on muskox populations is poor. *Teladorsagia boreoarcticus*, an abomasal nematode recorded in muskoxen, has been suggested to affect host body condition and reproduction (Kutz et al., 2004). Further identification of the strongyle-type eggs and abomasal nematode fauna is needed to reveal whether this parasite occurs in the Dovrefjell population, and the previous identification of *T. circumcincta* in this study population (Alendal and Helle, 1983) should be revisited, as *T. boreoarcticus* is morphologically very similar (Hoberg et al., 1999).

Infections with *Moniezia* in livestock are generally non-pathogenic. There is however some evidence for pathology in muskox (Samuel and Gray, 1974), with scouring reported.

Clinical disease associated with *Eimeria* infection is apparently uncommon in free-ranging Arctic ungulates (Kutz et al., 2012), but common in livestock with some *Eimeria* species. Diarrhoea due to coccidiosis is typically associated with young animals (Taylor et al., 2007). The highest oocyst excretion from muskoxen recorded in a survey in North America (Kutz et al., 2012) was 17,500 oocysts per gram (OPG) and our highest results were over twice this figure. The higher excretion rate in calves in our study is unsurprising, presumably reflecting lack of immunity but not

necessarily indicating clinical disease. It has been suggested that clinical disease would be associated with even higher excretion rates (over 800,000 OPG; Oksanen et al., 1990).

Each of these parasite infections, considered individually, may not have a significant impact on the health of the Norwegian muskox population. Nevertheless, widespread polyparasitism in conjunction with challenges due to other pathogens, climate change, and the fact that this population lives at the extreme southern edge of their climatic range, may mean that these animals are particularly vulnerable to synergistic negative effects. Comparison of our results with those from the earlier study of Alendal and Helle (1983) is complicated by the dissimilarity between study material and approaches (post mortem examination of muskoxen organs for helminths rather than examination of faecal samples for helminth eggs; no examination for intestinal protozoans). Nevertheless, it would seem that *Marshallagia* sp. and *Nematodirella* spp. may be less prevalent than during the 1970s. Possible reasons include interspecific competition, climate events, or reduced contact with domestic ruminants due to cessation of dairy farming in the region during the last thirty years. Muskoxen can act as reservoirs of parasitic infection for other animals sharing or encroaching upon their grazing areas and, in turn, be infected themselves. Alendal and Helle (1983) recommended that establishment of muskox populations should be restricted to areas of ample size and with as little contact with other ruminants as possible. Their recommendation was based on the apparent low resistance of muskoxen to parasite infection in their study. Subsequent demonstration of host switching of many parasites to muskoxen in areas of contact with wild and domestic caprines and cervids in North America underlines the importance of this recommendation (Hoberg et al., 2002; Kutz et al., 2012, 2013). The respiratory disease outbreaks from this population (Ytrehus et al., 2008; Norwegian Veterinary Institute, 2013) further highlights potential impacts from interactions between muskoxen and domestic animals.

## 5. Conclusions

Baseline studies on parasite fauna are essential for understanding impacts of climate change in host-parasite assemblages (Hoberg et al., 2003; Kutz et al., 2009). This study investigates an introduced muskox population which occurs in a region that exceeds thermal tolerances of this Arctic adapted species. Changes in faunal diversity have occurred subsequent to the first study looking at this same population although, given the different methodologies used, direct comparisons are challenging. Anthropogenic and climate-related breakdown of ecological barriers are common pathways for host switching episodes in modern times, expediting natural processes that shape our biota and host-parasite associations (Hoberg et al., 2012; Kutz et al., 2014). In the present study, sympatry with domestic and wild ungulates has facilitated such host-switching events, which translate into new host-parasite associations: *M. capillaris* and *C. xiaoi*. Previous disease outbreaks in this population further suggest its sensitivity to the influx of new pathogens and the potential for exacerbation by climatic perturbations. It is difficult to predict how this muskoxen population will respond to these combined pressures but the Dovrefjell population may serve as a sentinel for cold-adapted species in other regions and as such continued monitoring of this vulnerable population is of paramount importance.

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